

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

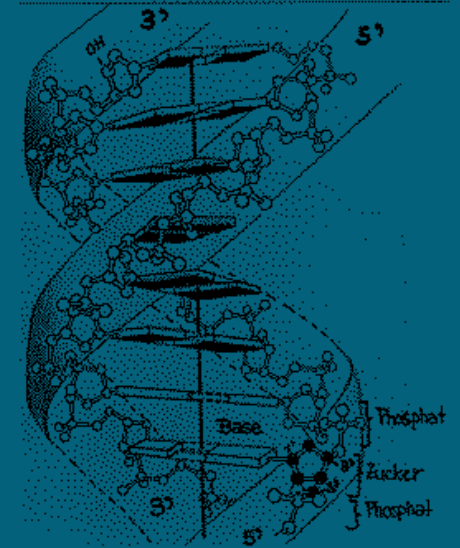
# Serologic Evaluation of Autoimmune C.T Diseases



By

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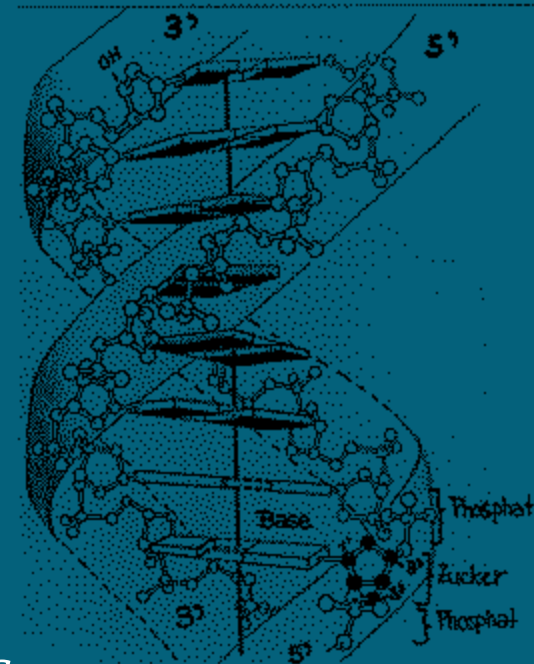
# Historical Perspective

- ❑ LE cell phenomenon 1940.
- ❑ Anti-phospholipid Abs 1952.
- ❑ Anti-DNA Abs 1957.
- ❑ Other auto-antibodies 1970s.
- ❑ (Sm ,Ro and La) RNP were named after the patients in whom they were first described.

- C.T. diseases are a group of autoimmune disorders that have overlapping clinical features
- Autoimmune C.T diseases:

### 1. LE :-

- Systemic
- Discoid
- Subacute
- Overlap of 2 or more LE subsets
- Overlap of LE with other C.T diseases



## 2. Scleroderma

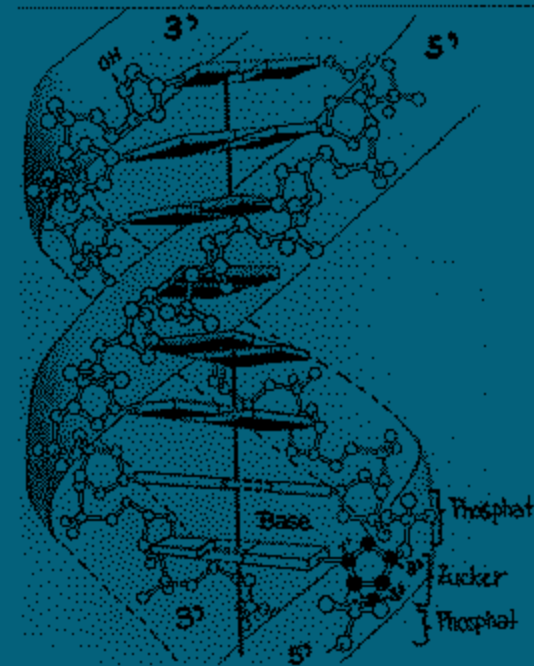
- Cut.
- Systemic
  - Limited (CREST syndrome)
  - Diffuse

## 3. Dermatomyositis

## 4. Sjogren's syndrome

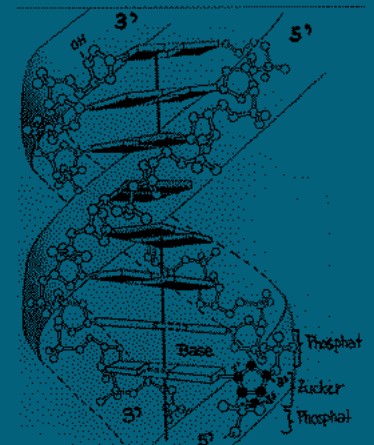
## 5. MCTD

## 6. Overlap C.T disease



*Accurate diagnosis of one of these disorders depends on (4 parameters):*

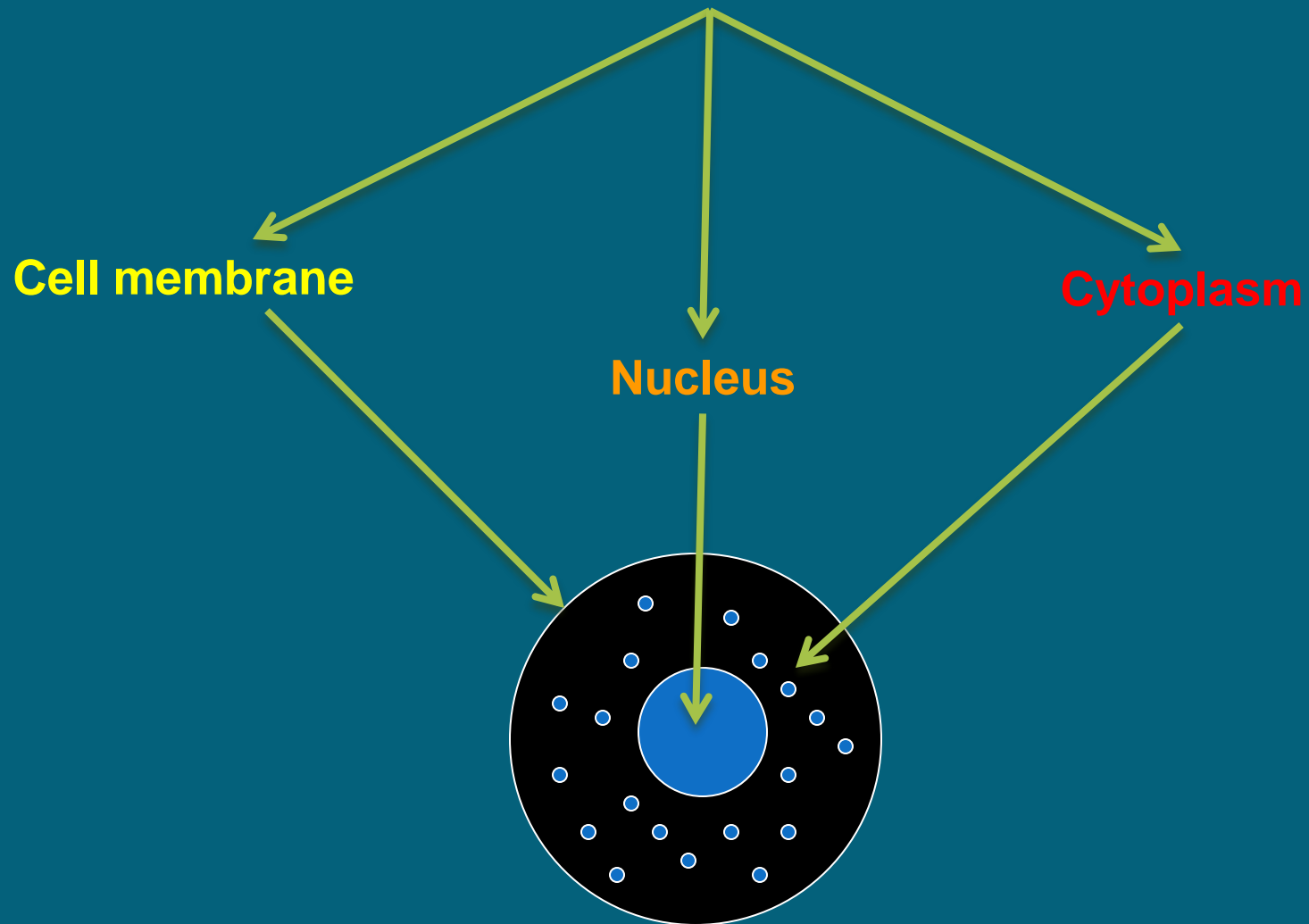
- Clinical findings
- Histopathology
- Tissue Immunofluorescence
- Serological testing



## N.B. :-

- Serological testing does not substitute for evaluation of other criteria
- Serological testing predicts prognosis e.g.
  - CDLE + high n DNA → SLE + cut. Involvement
  - CREST syndrome + anticentromere Abs → benign course

# Auto-antibodies





# Anti bodies in autoimmune CTDs:

## 1-Antibodies to DNA:

- ABDs to nDNA (dsDNA)
- Abs to SSDNA

## 2-Antibodies to small ribonucleoproteins:

- Anti- Ro (SS-A)
- Anti- LA( SS-B)
- Anti- Sm
- Anti U1 RNP

# Anti bodies in autoimmune CTDs:

3- Antibodies to histones.

4- Antibodies to centromere.

5- Antibodies to phospholipids.

# Serologic Evaluation

```
graph TD; A[Serologic Evaluation] --> B[Titre]; A --> C[Specificity];
```

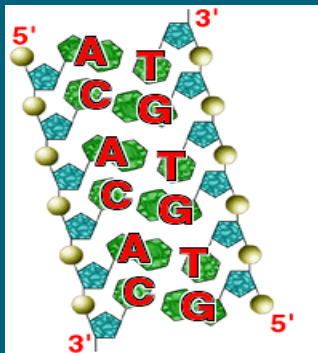
**Titre**

**Specificity**

# DNA antibodies

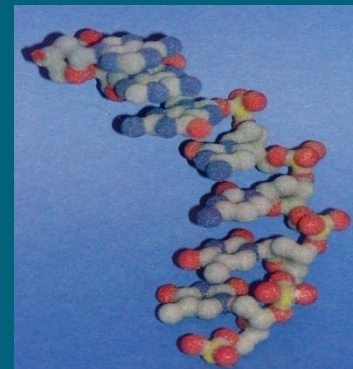
n DNA (ds DNA)

- **+ve 50%-83%**
- **(SLE)**
- **With high titre**



ss DNA

- **Very low diagnostic value**
- **(SLE)**



# Histone ABs

- Basic protein bind DNA helical structure
- Characteristic of *drug*-induced SLE (90%)
  - Tetracyclines
  - Griseofulvin
  - Hydralazine
  - PAS etc.



Histone Abs (30% ) in idiopathic SLE,

However

these patients have other  
anti nuclear Abs



## **Idiopathic LE**

**Histones Abs (30%)**

**Other autoantibodies +ve**

**Low complement level**

## **Drug induced LE**

**Histone Abs(90%)**

**-ve**

**Normal levels**

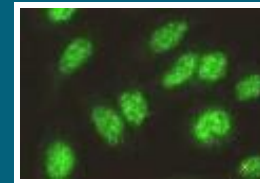
- Histone antibodies are considered as a hallmark of drug-induced LE. However, different drugs are capable of inducing different auto-antibodies rather than histone
- Griseofulvin, Terbinafine (anti-Ro )
- TNF alpha-inhibitors (anti-dsDNA)
- Minocycline, Methyldopa, Isoniazide ( Histone antibodies)



# RNP antibodies

## sRNP (RNA + protein)

- Anti-Ro (SS-A)
- Anti-La (SS-B)
- Anti-Sm
- U<sub>1</sub> RNP



## Anti-Ro (SS-A)

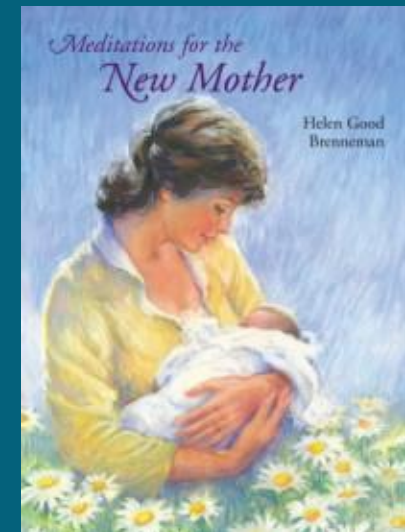
1. LE
2. Sjogren's syndrome

## Anti-La (SS-B)

1. LE
2. Sjogren's syndrome
  - Closely related to anti-Ro abs.
  - 90% of sera with anti-la also +ve for anti-Ro abs.

# Indications for ordering anti-Ro & anti-La Abs.

- Photosensitivity
- Neonatal LE
- Sjogren's syndrome
- SALE
- DLE + Photosensitivity
- Mother of infants with neonatal LE



# Anti Ro , Anti La

- The presence of Anti Ro and Anti La antibodies in pregnant woman with or without a full blown picture of autoimmune disease conveys a significantly increased risk of the neonatal lupus syndrome.
- Patients with SLE and anti-La antibodies have a lower incidence of renal disease.

## Abs to U<sub>1</sub> RNP

- MCTD (100%)
- SLE (30%) + other auto Abs
- Rare neonatal LE

## Abs to Sm

- SLE only (15- 40%)
- Most patients with Sm Abs have Abs to U<sub>1</sub> RNP
- The converse is not true

*U<sub>1</sub> RNP confirm MCTD*

*Sm confirm SLE*

# Anti- ribosomal Abs

- Anti- cytoplasmic Abs.
- 10-40% of LE patients.
- One of **diagnostic marker** in LE.
- They are frequently found in patients with **behavioral disturbances**.

# Anti-Ku

- Ku- it binds to termini of dsDNA.
- It plays a role in DNA replications, and repair.
- Evidence of its binding to **human** rather than **mouse** antigen has been reported.
- It is found in scleroderma-myositis overlap, scleroderma and in SLE

# Other Auto-Antibodies

- Scl-70 Abs (SSc)
- Anti-centromere Abs (CREST)
- Jo-1( Anti-synthetase syndrome)
- Mi-2 ( Classic dermatomyositis)



# Flourescent ANA test

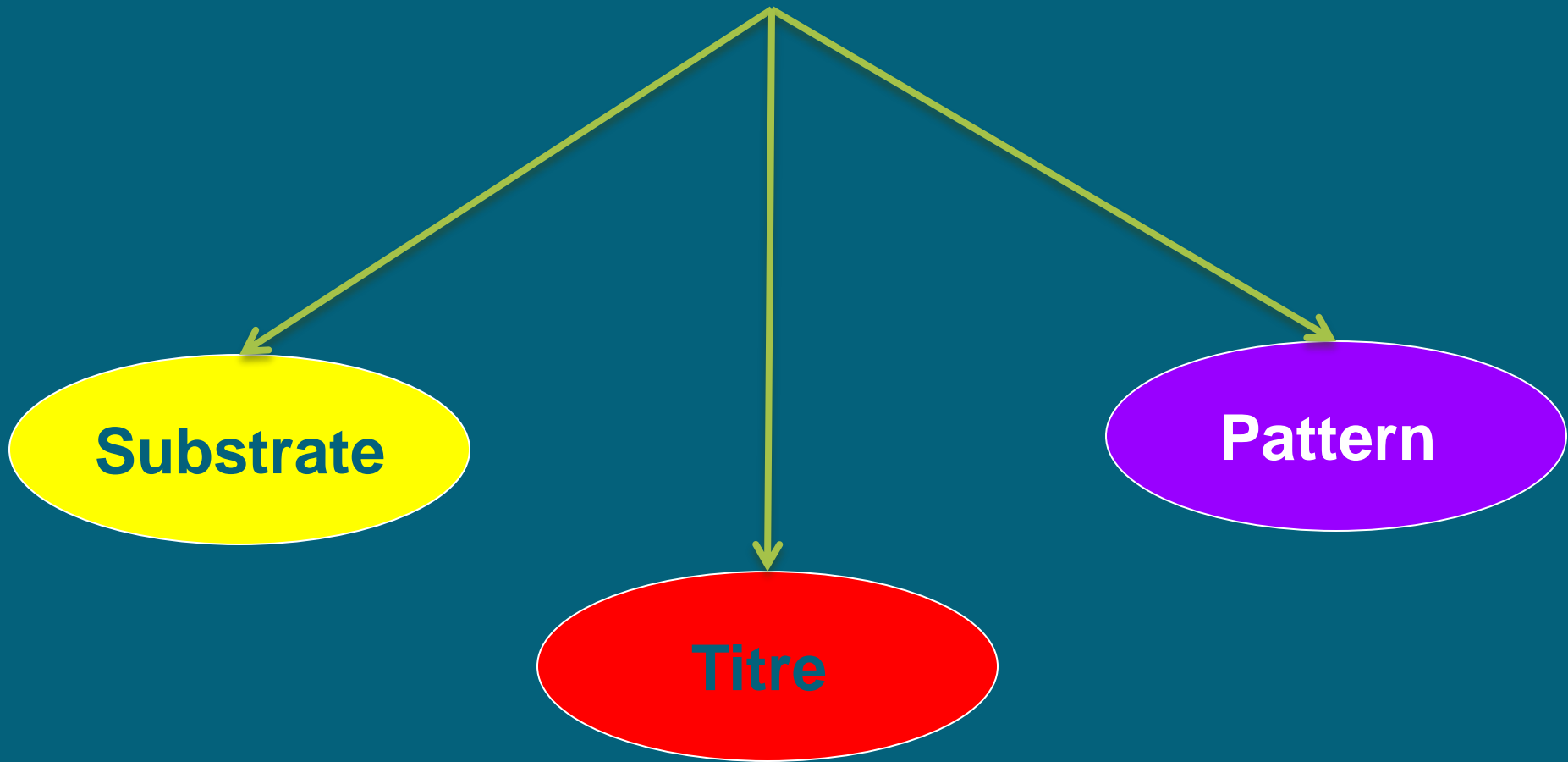
- Screening test
- Indirect IF test

## Indications:

1. CDL
2. Suspicion of CTD
3. Phototherapy
4. Chronic vasculitis



# Interpretation of ANA results



# Substrate

```
graph TD; Substrate --> Animal; Substrate --> Human; Animal --> Minus; Minus --> Plus; style Animal fill:#00FFFF,stroke:#000,stroke-width:1px; style Human fill:#FF00FF,stroke:#000,stroke-width:1px; style Minus fill:#FFFF00,stroke:#000,stroke-width:1px; style Plus fill:#FFFF00,stroke:#000,stroke-width:1px;
```

## **Animal**

- Mouse kidney
- Rat liver

## **Human**


- HEp-2 cells
- More sensitive

**-ve**



**+ve**

# Substrate

Animal (ANA -ve LE)  Human (ANA +ve LE)

- ANA -ve LE :

(most of such patients have anti -Ro Abs on HEP -2cell)

Is ANA –ve LE is due to animal  
substrate only?



# No

## Another reason

- In patients with SLE if patient's ANB Abs are solely directed against ssDNA, as the fluorescent ANA substrate has intact nuclei without single strands of DNA.

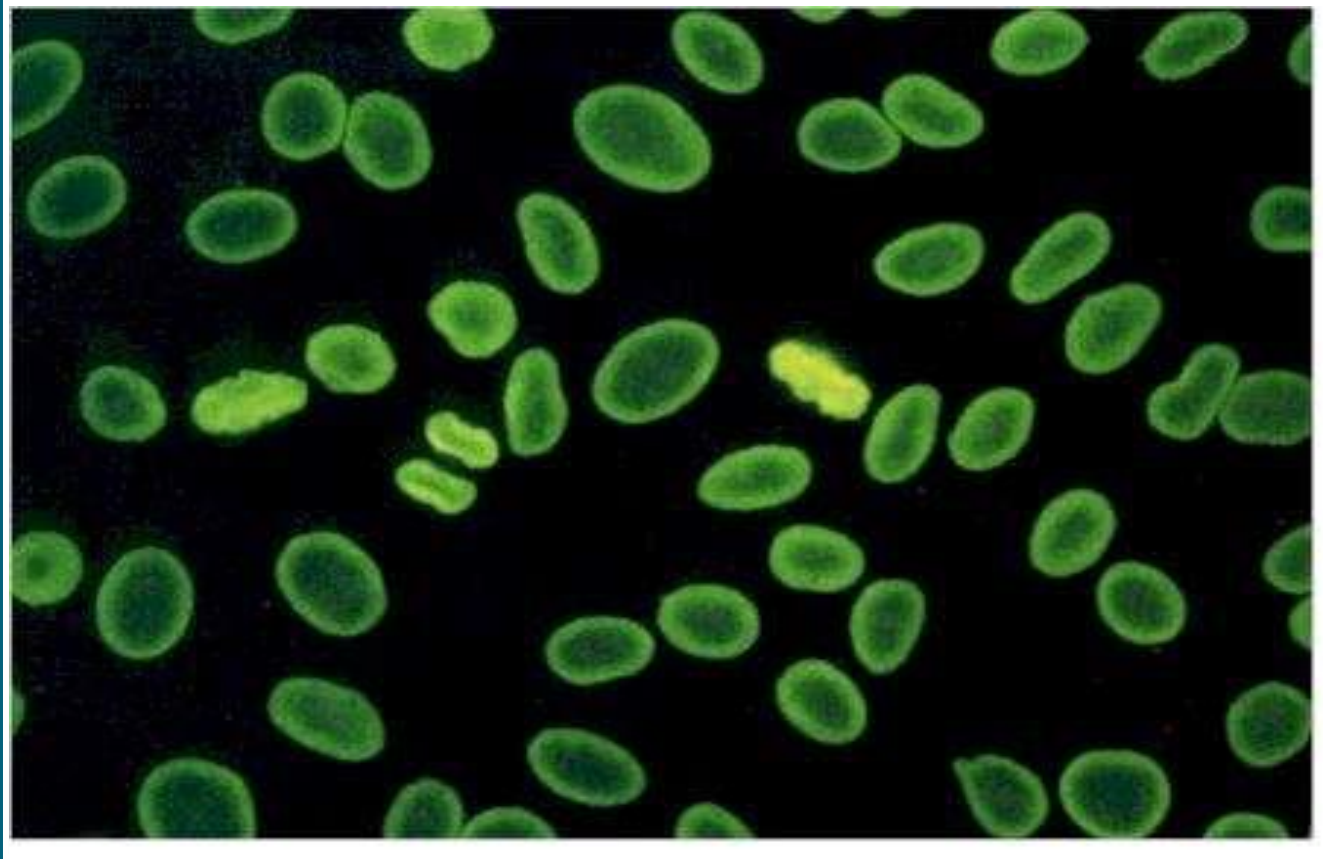
**SO:** extracted nDNA are denatured to produce ssDNA.

# Titre

- 1:80 or less → not diagnostic
- 1:160-1:320 → diagnostic
- High titre can occur in healthy persons:
  - 1:160 (5%)
  - 1:320 (3%)

So, diagnosis of CTDs should not be made solely on titre of an ANA test

# Patterns



PERIPHERAL



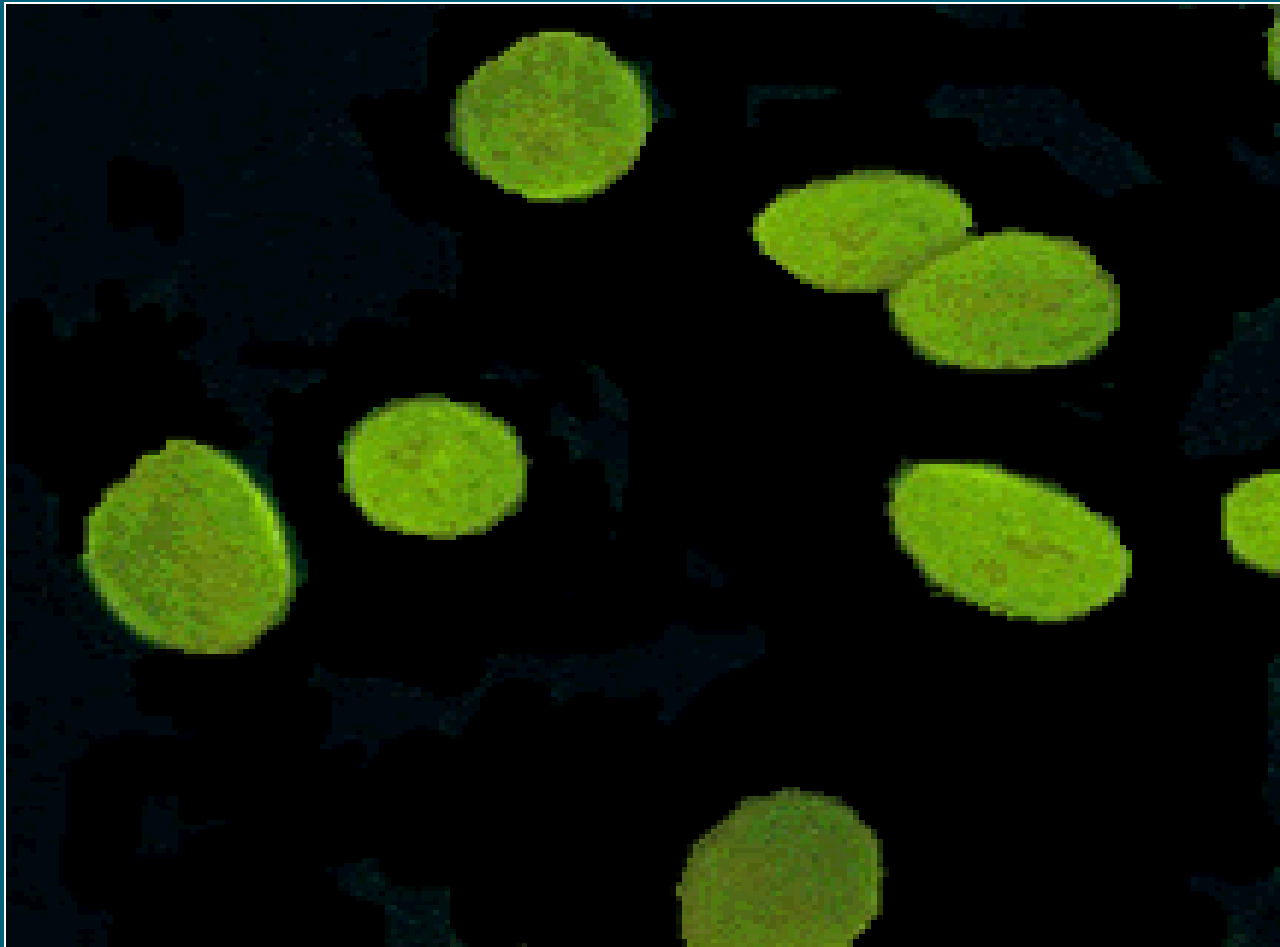
nDNA



SLE

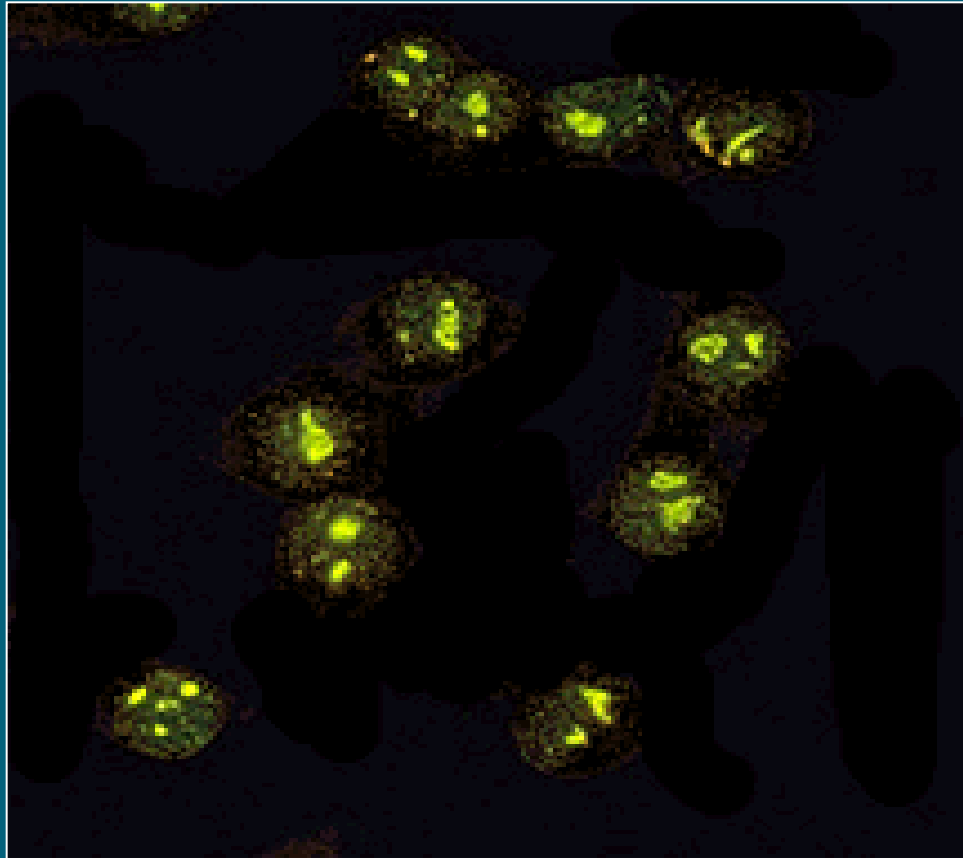


# Patterns



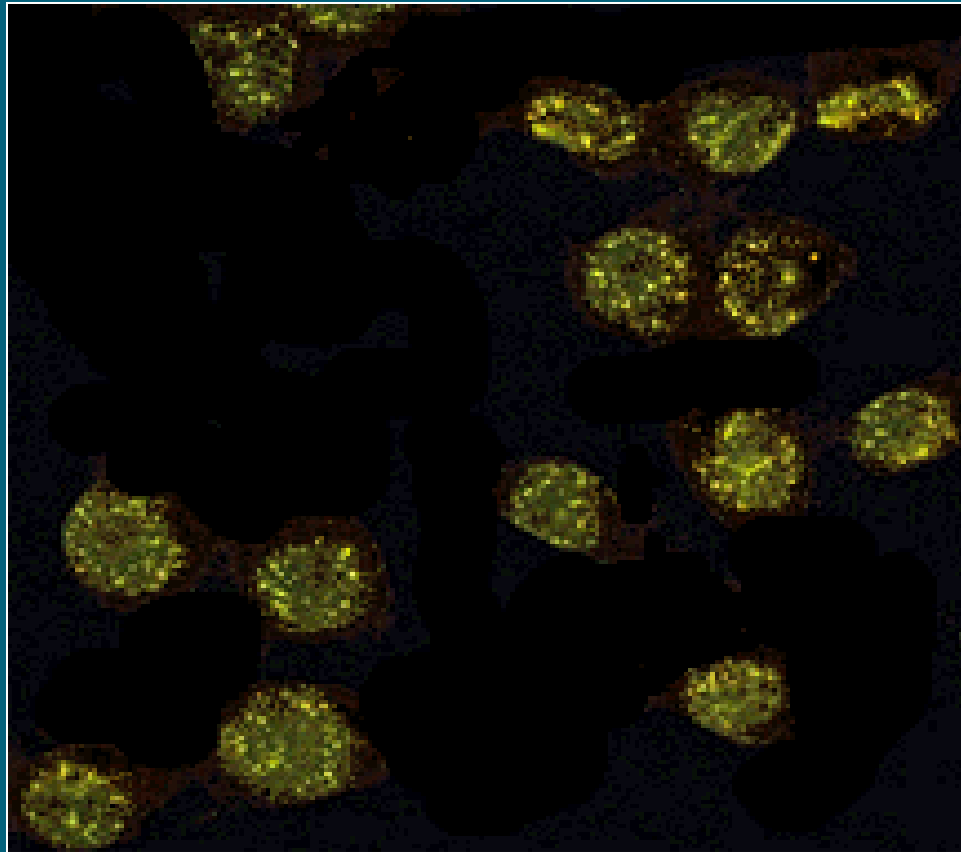
Homogenous → nDNA → SLE

# Patterns



Nucleolar → RNP → SSc & LE

# Patterns



**SPECKLED** → **Various RNP** → **MCTD, SLE, Sjogren's**

# Patterns

<b>Peripheral</b>	<b>nDNA</b>	<b>SLE</b>
<b>Homogenous</b>	<b>nDNA</b>	<b>SLE</b>
<b>Nucleolar</b>	<b>RNP</b>	<b>SSc &amp; LE</b>
<b>Speckled</b>	<b>Various RNP</b>	<b>MCTD, SLE, Sjogren's s.</b>

# Serologic profiles in CTDs

[illegible]

# Antiphospholipids Abs (APAs)

- Indications for APA testing:
  - Lividoreticularis
  - Purpura & necrosis
  - Internal organ thrombosis
  - Recurrent miscarriages
  - Screening in patients with SLE



Пурпура. Множественные кровоизлияния в кожу нижних конечностей

# Conclusions

- -ve nDNA doesn't exclude SLE
- -ve ANA on animal substrate must be done on human substrates.
- Sm Abs is a diagnostic and unique marker for SLE.
- Detection of Sm , anti -dsDNA, anti ribosomal Abs are strongly support clinical diagnosis of SLE.
- Sm Abs & U1 Abs (ENA) Differentiated from each other by using Ribonuclease enzyme.(U1 is ribonuclease sensitive and Sm is resistant)

# AL-AZHAR UNIVERSITY

# THANK YOU